Presentation Name

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Drug Metabolizing Enzymes and Pharmacogenomic Testing:
A Joint FDA/Johns Hopkins/PhRMA Workshop

Special Considerations for Individual Metabolic Biomarkers: CYP2C9

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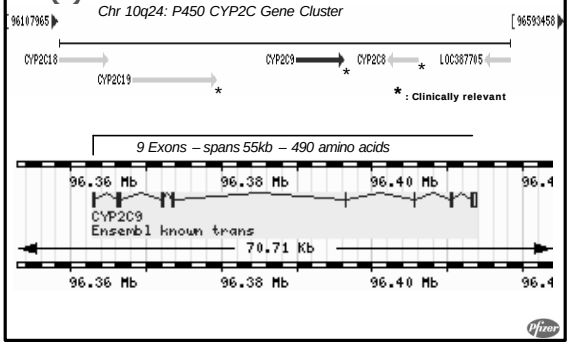
1

Presentation Overview

- The gene - The protein
- Genetic variation of CYP2C9
- *In vitro* and *in vivo* correlation
- *In vivo* genotype/phenotype correlation
- Defining "metabolic status"
- Distinguishing features is different ethnic groups
- Summary with respect to clinical programs

Genomic Structure for CYP2C9

Chr 10q24: P450 CYP2C Gene Cluster



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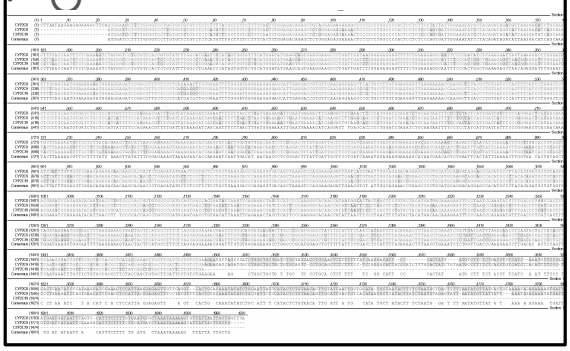
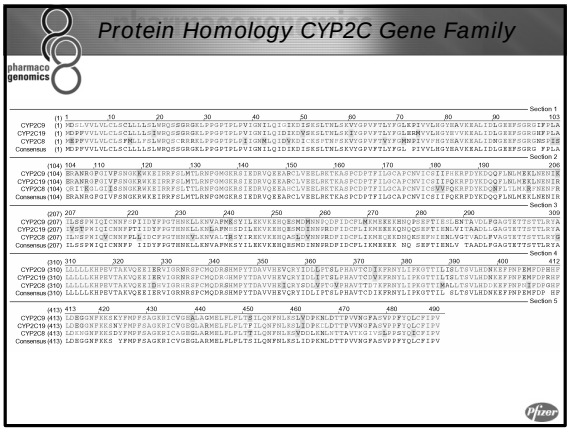
DNA Homology CYP2C Gene Family



Protein Homology CYP2C Gene Family



Role of CYP2C9

- Primary CYP2C in liver, appr. 20% of hepatic content
- Liver microsomal monooxygenase, localized in ER
- Major role in drug metabolism with numerous substrates including:
  - Antidiabetic therapies – glipizide, tolbutamide
  - Anticonvulsants – phenytoin
  - Angiotensin II receptor antagonist – losartan
  - HMG CoA Reductase Inhibitor – fluvastatin
  - NSAIDs

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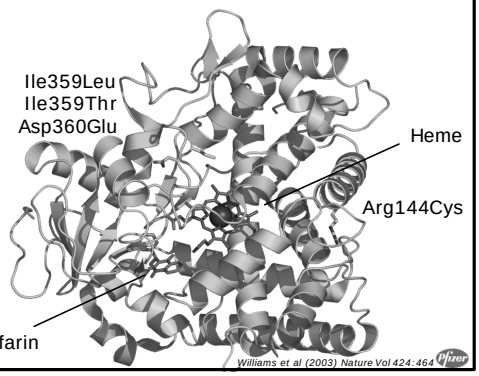
Coding Variation of CYP2C9

	Allele	Effect of Change	Protein Function
Most Common Variants	CYP2C9*1	Wild Type	Active Enzyme
	CYP2C9*2	Arg144Cys	Intermediate
	CYP2C9*3	Ile359Leu	Higher Km/altered Vmax
Rare Variants	CYP2C9*4	Ile359Thr	
	CYP2C9*5	Asp360Glu	
	CYP2C9*6	DelAden818 Leu208Val	Lack of function
	T/C transition Intron 2		

Structure of CYP2C9



Williams et al (2003) Nature Vol 424:464

Demonstration of CYP2C9 Activity in vivo and in vitro

Human Liver Microsomes and Recombinant CYP2C9 Demonstrate Specificity for Phenytonin and Tolbutamine Metabolism

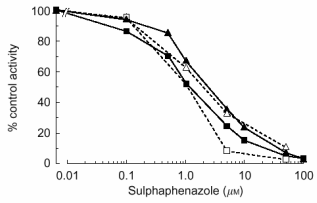


Figure 2 Sulphaphenazole inhibition of phenytoin and tolbutamide hydroxylation in human liver microsomes (HLM) and COS cells transfected with a *CYP2C9* (wild type) cDNA. □—□ phenytoin, HLM; ■—■ phenytoin, CYP2C9; △—△ tolbutamide, CYP2C9; ▲—▲ tolbutamide, HLM. The concentrations of phenytoin and tolbutamide were 150 μM and 1000 μM, respectively. Data from references 15 and 30.

Reviewed in Miners and Birkett (1998) Br J Clin Pharmacol 45: 525-538.

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Demonstration of CYP2C9 *1 and *3 in vitro Activity

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*CYP2C9 *3 reduced 6- and 7-hydroxylation of S-warfarin*

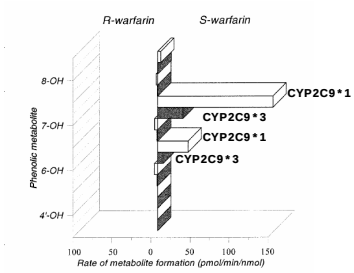

FIG. 5. Metabolite profiles generated by CYP2C9WT (open bars) and CYP2C9V1 (solid bars) from (*R*)- and (*S*)-warfarin. Standard incubations were carried out with 650 μM (*R*)- or (*S*)-warfarin. Samples were spiked with deuterated warfarin phenol internal standards prior to extraction, derivatization, and quantitative analysis by GC/MS as described under Materials and Methods.

Haining et al (1996) Arch Biochem Biophys 333: 447-458.

Altered Kinetic Properties of CYP2C9 *3

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Table 1. Kinetic parameters of piroxicam 5′-hydroxylation, phenytoin 4′-hydroxylation, tenoxicam 5′-hydroxylation, mefenamic acid 3′-methylhydroxylation, tolbutamide methylhydroxylation, *S*-warfarin 7-hydroxylation and diclofenac 4′-hydroxylation by wild-type CYP2C9 (Ile^{359}) and its Leu^{359} variant expressed in yeast

	K_m (μM)	V_{max} (*pmol/min/pmol P450*)	V_{max}/K_m ($\mu l/min/nmol$ *P450*)
Piroxicam 5′-hydroxylation			
Ile^{359}	40 ± 3	0.408 ± 0.026	10.2 ± 0.6
Leu^{359}	61 ± 33	0.019 ± 0.006***	0.3 ± 0.1***
Phenytoin 4′-hydroxylation			
Ile^{359}	15 ± 4	0.191 ± 0.018	14.0 ± 4.8
Leu^{359}	30 ± 7*	0.015 ± 0.003***	0.5 ± 0.2*
Tenoxicam 5′-hydroxylation			
Ile^{359}	28 ± 3	0.264 ± 0.003	9.44 ± 0.81
Leu^{359}	90 ± 19*	0.034 ± 0.014***	0.41 ± 0.21***
Mefenamic acid 3′-hydroxylation			
Ile^{359}	8.4 ± 0.5	14.9 ± 3.0	$1.80 \times 10^3 \pm 0.42 \times 10^3$
Leu^{359}	40.8 ± 7.5*	4.2 ± 1.4**	$0.10 \times 10^3 \pm 0.01 \times 10^3$*
Tolbutamide hydroxylation			
Ile^{359}	151 ± 32	9.2 ± 1.0	61.4 ± 6.1
Leu^{359}	1729 ± 512*	10.0 ± 2.0	5.9 ± 0.7***
S-warfarin 7-hydroxylation			
Ile^{359}	5.8 ± 0.8	0.248 ± 0.018	43.7 ± 7.0
Leu^{359}	21.6 ± 1.5***	0.111 ± 0.012***	5.1 ± 0.6*
Diclofenac 4′-hydroxylation			
Ile^{359}	3.9 ± 0.3	35.6 ± 1.3	$9.16 \times 10^3 \pm 0.29 \times 10^3$
Leu^{359}	12.6 ± 2.8*	33.3 ± 2.6	$2.69 \times 10^3 \pm 0.76 \times 10^3$***

The data are shown as the mean $\pm$ SD of three different experiments. * $P<0.05$, ** $P<0.01$ and *** $P<0.005$ compared with Ile^{359}.

Takanashi et al (2000) Pharmacogenetics 10: 95-104.

Summary of In vitro Studies

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CYP2C9 allelic variants exhibit differing affinity (Km) and/or instrinsic clearance (Vmax/Km) for differing substrates

Examples:

*CYP2C9*2*
- impaired 6-/7-hydroxylation of s-warfarin
- small if any effect in Vmax for tolbutamide
- no effect on methyl hydroxylation of torsemide

*CYP2C9*3*
- reduced catalytic activity across all *CYP2C9* substrates
- lower maximium catalytic rate and/or lower affinity for s-warfarin, tolbutamide, phenytoin
- *3 homozygotes possess significant impairment in substrate metabolism

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Warfarin Studies Demonstrate In vivo Genotype Effects

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Table 3. Prescribed Daily Dose of Warfarin in Relation to CYP2C9 Genotype*

	Genotype					
	*1/*1	*1/*2	*1/*3	*2/*2	*2/*3	*3/*3
No.	127	28	18	4	3	5
Daily maintenance warfarin dose, mg						
Mean (SD)	5.63 (2.56)	4.88 (2.57)	3.32 (0.94)	4.07 (1.48)†	2.34 (0.35)	1.60 (0.81)
Median (IQR)	5.27 (3.93-7.14)	4.64 (3.61-5.29)	2.92 (2.50-3.93)	3.86 (2.50-4.00)	2.32 (2.00-2.70)	1.61 (1.14-1.96)

*Kruskal-Wallis $\chi^2_4 = 37.348$; $P<.001$. IQR indicates interquartile range.
†Mean doses (mg) for the 4 patients were 2.50, 3.71, 4.00, and 6.07. The mean of these is 4.07; however, the 75th percentile is 4.00. The patient with the mean daily dose of 6.07 had a prosthetic valve and experienced a serious bleeding event. This reflects the potential skewness that data from patients with prosthetic valves can introduce to small samples and reflects the range of maintenance doses that can occur in a clinical setting. An analysis was performed in which 12 patients having prosthetic valves with a higher target international normalized ratio range (2.5-3.5) were excluded; the effect on hazard ratio estimates was trivial (see text).

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Higashi et al. (2002) JAMA 287: 1690-1698.

CYP2C9 Genotypes and Clinical Outcomes

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Table 5. Unadjusted and Adjusted (for Warfarin Daily Dose) HRs for Clinical Outcomes in Patients Having the CYP2C9 Variant Genotype*

	HR (95% CI)	
End Point	Unadjusted	Adjusted†
Time to therapeutic INR	0.91 (0.65-1.27)	0.95 (0.68-1.34)
Time to above-range INR	1.28 (0.94-1.74)	1.40 (1.03-1.90)
Time to stable dosing	0.60 (0.42-0.85)	0.65 (0.45-0.94)
Time to bleeding event		
Initiation phase (first 3 mo)	3.94 (1.29-12.06)	NA
Entire follow-up period	2.39 (1.18-4.86)	NA

*Excluding 12 patients with prosthetic valves, the hazard ratios (HRs) for time to therapeutic international normalized ratio (INR) were 0.99 (95% confidence interval [CI], 0.71-1.38); time to above-range INR, 1.48 (95% CI, 1.04-2.11); time to stable dosing, 0.59 (95% CI, 0.40-0.85); and time to first serious/life-threatening bleeding event, 2.54 (95% CI, 1.18-5.46). NA indicates not applicable (no covariates were included in the final model because they did not result in a >5% change in HR of bleeding risk [see text]).
†Warfarin daily dose was the only covariate used in this adjustment of the HR.

- *CYP2C9 gene variation has potential to influence over anticoagulation and bleeding events*
- *Caucasians and Asians exhibit differing maintenance doses*

Higashi et al. (2002) JAMA 287: 1690-1698.

Ethnic Distribution of CYP2C9 *2 and *3 Variants

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TABLE 1 Ethnic distribution of the *CYP2C9* allelic variants[a]

	Cys^{144} (*2)		Leu^{359} (*3)		
Ethnicity	**n**	**%**	**n**	**%**	**References**
Asians					
Chinese	466	0.0	426	2.1	24, 48
Japanese	1394	0.0	1394	1.9	18, 42, 44, 49, 50[b]
Korean	1148	0.0	1148	1.1	47a
Total	3008	0.0	2968	1.6	
Blacks					
American	1098	2.9	500	0.8	24, 51[b]
Caucasians					
American	370	10.0	1512	7.9	18, 24, 51[b]
British	588	14.1	400	9.5	28, 38, 52
German	988	11.3	734	7.8	53, 54
Swedish	860	10.7	860	7.4	55
Turkish	998	10.6	998	10.0	26
Total	3804	11.3	4504	8.4	

[a]n, combined number of the total alleles tested; %, percent of the allelic variants.
[b]UI Schwarz, EF Choo, GK Dresser, CM Stein, AJJ Wood, DM Roden, GR Wilkinson, RB Kim. 2000, unpublished data.

Xie et al (2001) Annu. Rev. Pharmacol. Toxicol. 41: 815-850.

Presentation Name

Variations in CYP2C9 Regulatory Region

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Regulatory region variation may contribute to metabolism heterogeneity

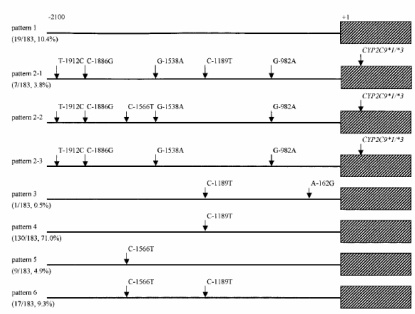

Fig 2. Sequence patterns in 5´-flanking region of human *CYP2C9* gene among 183 Japanese subjects. Frequencies of each pattern are presented in *parentheses*.

Shintani et al (2001) Clin Pharmacol Ther 70:175-82.

Functional Characterization of Regulatory Variation

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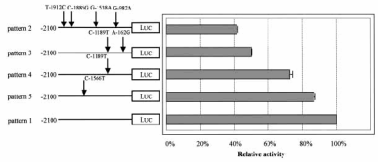

Fig 3. *CYP2C9 5´*-flanking region reporter gene constructs *(left)* and their respective luciferase activities in HepG2 cells *(right)*. Luciferase activity in pattern 1 (without mutation) is expressed as 100%. Data are presented as mean ± SD of 3 experiments.

Shintani et al (2001) Clin Pharmacol Ther 70:175-82.

Summary and Clinical Implications

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- CYP2C9 represents the predominant CYP2C protein in liver
- CYP2C9 substrates can be identified through *in vitro* assessments
- Functional genetic variants within the *CYP2C9* coding region (*2, *3) may be critical to assess in clinical studies involving CYP2C substrates
- *2 and *3 alleles appear to ascertain the major gene variation, however, outliers could be examined for rare alleles including *4, *5, *6 or through comprehensive gene resequencing to identify novel gene variants
- 5' promoter variation may contribute additional human genetic heterogeneity